J Endocrinol Reprod 11 (2007) 2: 62 - 68

Original Article

Influence of coconut husk retting effluent on metabolic, interrenal and thyroid functions in the air-breathing perch, *Anabas testudineus* Bloch

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Summary

To understand the physiological mechanism of stress tolerance in fish living in coconut husk retting grounds, we examined the metabolic pattern, and interrenal and thyroidal activities in the air-breathing perch, *Anabas testudineus* after exposing them to the effluent of coconut husk retting (CHRE). Cortisol, the end product of interrenal axis and triiodothyronine (T_3) and thyroxine (T_4), the primary hormones of thyroid, were measured in the plasma of these fish together with the indices of metabolic regulation. Five days of CHRE exposure increased the plasma cortisol but decreased the plasma T_4 without affecting the plasma T_3. The concentration of plasma glucose, triglycerides and urea were significantly increased in the CHRE-exposed fish. Significant reduction in the concentration of liver total protein, RNA and DNA occurred in the CHRE-treated fish. CHRE treatment, while increasing the alanine aminotransferase and alkaline phosphatase activities, decreased the aspartate aminotransferase activity in the liver. Besides identifying plasma glucose and cortisol as reliable biomarkers of CHRE-induced stress, our results suggest that these fish reallocate their energy resources during stress where both interrenal and thyroid glands have roles to play.

Key words: Teleost fish, stress, thyroid hormones, intermediary metabolism, coconut husk retting

Introduction

Cortisol, the end product of corticosteroidogenesis in the interrenal cells located in the head kidney, has been associated with teleostean metabolic and hydromineral regulations (Vijayan et al., 1991, 2003; Dang et al., 2000). This primary stress hormone in teleost fishes stimulates the synthesis of energy substrates (Vijayan et al., 1997) and thus influences the energy metabolism of fish during stress (Wendelaar Bonga, 1997; Peter, 2007).

Similar to interrenals, fish thyroid is also involved in the regulation of a wide range of biological processes including metabolism and osmoregulation (Leatherland, 1994; Power et al., 2001; Peter et al., 2000, 2007). Besides being sensitive to many natural environmental variables (Specker, 1988; Leatherland, 1994), thyroxine (T_4) and triiodothyronine (T_3), the primary hormones of the thyroid (THs), are also known for their role in regulation of metabolism in fish. For example, THs have been shown to affect intermediary metabolism in climbing perch (Peter, 1996; Nair and Oommen, 1998; Varghese et al., 2001). Disturbances in the metabolic regulation during stress have been reported in fishes including climbing perch (Sumpter, 1997; Peter et al., 2007). However, the mechanism of thyroidal response to environmental toxicants is not known (Brown, 1993; Wendelaar Bonga, 1997; Peter et al., 2004, 2007).

Fishes rely on complex neuro-endocrine mechanisms to tackle the effects of stressors of varied origin. For coping with stressful challenges, they need to reallocate the rate of energy utilization in order to maintain the physiological homeostasis (Lawrence et al., 2003; Peter, 2007). Alterations in energy metabolism, one of the main outputs of secondary stress response (Barton and Iwama, 1991), could thus be immediately beneficial to stressed fish. Likewise, the availability of THs to various tissues is well regulated in the event of stressful challenges (Leji et al., 2007). The varied responses of plasma THs to stressors appear to be dependent on the type of imposed stressor (Vijayan et al., 1997).

The role of THs in intermediary metabolism of stressed fish has not been addressed adequately (Leji et al., 2007). It is likely that the compensatory adaptive modifications occurring in the metabolic or osmoregulatory machinery of stressed fish may require support of THs or cortisol or both (Peter et al., 2004; Peter, 2007). We, therefore, analyzed the metabolic indices, and interrenal and thyroid functions in the fish, climbing perch, after exposing them to the coconut husk retting effluent (CHRE),

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a mixture of toxicants released during the retting process of coconut husk (Peter et al., 2007).

Materials and Methods

Fish

The air-breathing perch *Anabas testudineus* is a native teleost fish in the back-waters of Kerala in Southern India. Adult perch of both sexes weighing 45-50 g were maintained in large tanks and fed once a day with 1.5% body weight commercial fish feed. Before commencement of the experiment, the fish, which were in the pre-spawning phase (April-May), were transferred to glass aquaria (45 L) and kept for two weeks at water temperature 28 ± 1°C and photoperiod 12 h L: D cycle. Feeding was stopped 24h prior to sampling.

Protocol

Thirty-two fish were divided into 4 groups of eight each and placed in separate glass aquaria. The fish of group 1 were freshwater control and group 2 fish were exposed to lake water for five days. Each fish in groups 2 and 3 was treated with CHRE at concentration 1:9 and 1:4, respectively, for five days. The aquaria water was slowly replenished with water of same dilution and no mortality was recorded. All treatments were done concurrently to avoid interaction with the other environmental variables. Strict care was taken to minimise stress to the fish during the experiments.

Sampling and analyses

Five days after CHRE exposure, blood was drawn by caudal puncture and the fishes were sacrificed by decapitation. Blood plasma was collected after centrifugation (1500 x g for 10 min). The liver was quickly removed, weighed and stored in storing buffer at -20°C. A 10% liver homogenate was prepared in cold 50 mM imidazole-HCl buffer (pH 7.4), which was then centrifuged at 700 x g (4°C) for 10 min and the supernatant was stored at -20°C until analysis.

The concentrations of glucose, triglycerides and urea in the plasma and the activities of aspartate aminotransferase (AST, L-aspartate 2-oxyglutarate aminotransferase EC 2.6.1.1), alanine aminotransferase (ALT, L-alanine 2-oxyglutarate aminotransferase EC 2.6.1.2) and alkaline phosphatase (AlP, orthophosphoric-monoester phosphohydroxylase, alkaline optimum EC 3.1.3.1) in the liver were determined at 28°C in a Vital Lab auto-analyzer adopting standard procedures. Supplier's instructions with regard to pH, incubation time and temperature specified for individual enzymes were strictly followed (E. Merck-India Ltd, Mumbai) and reported elsewhere (Peter et al., 2007). Part of liver tissue was homogenized in 5 vol (w/v) $HClO_4$ and total protein (Folin *et al*., 1969), RNA (Mejbaum, 1959) and DNA levels (Burton, 1956) were determined.

Plasma T_3 and T_4 levels were measured adopting enzyme immunoassay (EIA) technique based on magnetic solid phase separation (Serozyme, Guidonia Montecelio, Italy). The EIA results were consistent with the T_3 and T_4 levels reported for this species (Peter et al., 2007).

Plasma cortisol was determined as described earlier (Peter, 2007) using a commercially available antibody, with some minor modifications of the manufacturer's protocol (Campero Scientific). In a duplicate experiment the total plasma cortisol was quantified adopting an ELISA method with a commercial cortisol kit (DiaMetra, Folgno, Italy, Catalog No. DKO 001) and the values revealed the same pattern of response.

Statistics

Data were statistically analysed for one-way analysis of variance supplemented by SNK test (Graphpad software). Statistical significance was accepted if $P<0.05$. The values are depicted as mean ± SEM (n=8).

Results

Exposure of perch to CHRE disrupted the pattern of metabolites and elevated plasma cortisol to significant levels, revealing a classic stress response in this fish. Significant increase in the concentrations of plasma glucose, plasma triglycerides and plasma urea ($P<0.05$; Fig. 1) were obtained after CHRE treatment. The concentration of total liver protein decreased significantly ($P<0.05$; Fig. 2) in the CHRE-exposed fish. Neither liver RNA nor liver DNA showed any change in the CHRE-treated fish (Fig. 2). CHRE exposure significantly ($P<0.05$) increased plasma cortisol and produced a significant ($P<0.01$) decrease in the plasma T_4, without affecting the plasma T_3 levels (Fig. 3). The AST activity in the liver decreased to significant levels ($P<0.05$) but ALT ($P<0.01$) an AlP activities in the liver were increased ($P<0.05$) (Table 1).

Table 1 Levels (IU/g) of AST, ALT and AlP in the liver of climbing perch treated with CHRE for five days. Each value is mean ± SE of eight fish

Status	*AST*	*ALT*	*AlP*
FW Control	525±11.4	48.9±2.9	10.43 ±0.9
LW Control	540±12.6	55.4±2.4	12.46±1.1
CHRE (1:9)	479±11.9	71.3±2.6*	22.16±1.8*
CHRE (1:4)	413±12.6*	76.4±3.1**	19.13±1.1*

* $P<0.05$, ** $P<0.01$

Discussion

CHRE exposure altered the metabolic pattern and activated the interrenal axis in climbing perch, suggesting that the fish were under stress. The elevated plasma glucose, an indicator of sympathetic activation during stress (Randall and Perry, 1992), seen along with the increased plasma cortisol, clearly indicates the classic stress response in the CHRE-treated fish. It is likely that the hyperglycemic effect of CHRE may be due to increased catecholamine and cortisol secretions as these hormones play significant glycolytic role in fish especially during stress (Vijayan et al., 1997). Glycogenolysis and subsequent hyperglycemia are the well documented responses in fish to various pollutants, revealing a toxic stress condition in fish (Li, 1996; Peter et al., 2004, 2007). Hyperglycemia may indicate an elevated energy demand, favouring oxidation of glucose resulting from the increased glycogenolysis in these fishes. This reflects an increase in the metabolic costs required to maintain homeostasis, implying that any disturbance in the homeostasis brings about serious consequences in the energy balance and, thus, for the growth and survival of the fish.

The elevated plasma cortisol and reduced plasma T_4 in response to CHRE exposure highlight the involvement of interrenal and thyroid in the CHRE-induced stress tolerance. Evidence is, thus, presented that these endocrines have an interaction, particularly on metabolic regulation. It is well known that stressors influence the interrenal and thyroid functions in fish. For example, exposure of catfishes *Heteropneustes fossilis* and *Clarias batrachus*, to malathion and endosulfan caused changes in circulating THs (Yadav and Singh, 1986; Sinha et al., 1991). A decrease in T_3 level has been reported in rainbow trout exposed to acidic water (Brown et al., 1990) and to starvation (Oommen and Matty, 1991). On the contrary, an activated thyroid axis was found in the climbing perch after kerosene treatment (Peter et al., 2007)

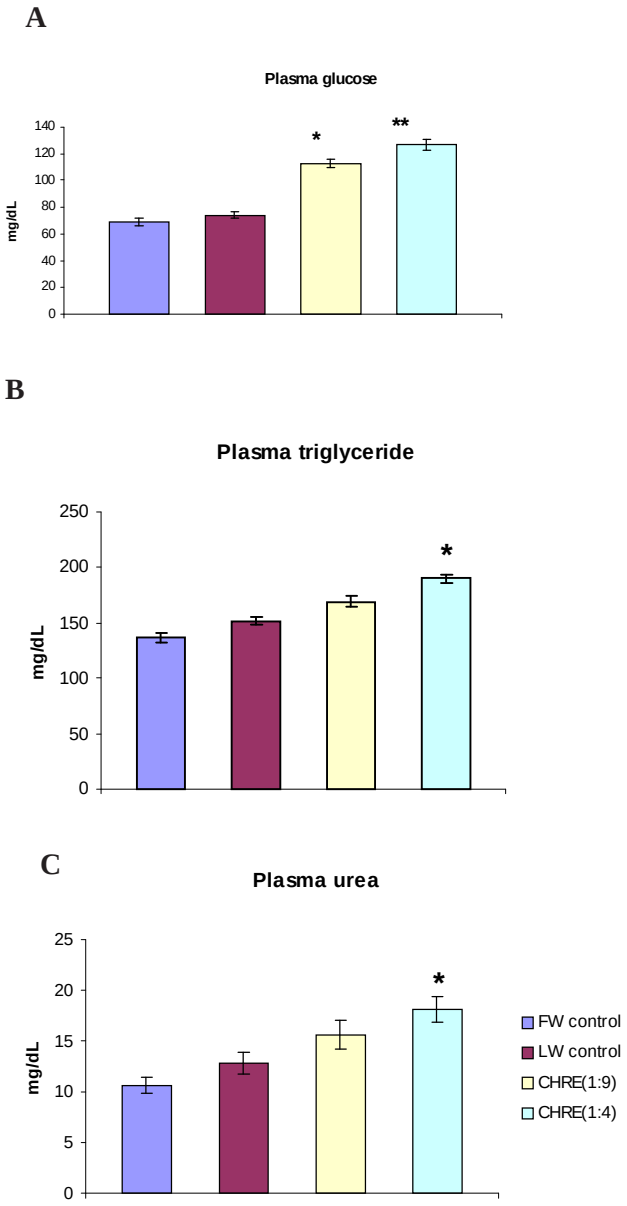

Fig. 1. Plasma glucose (A), plasma triglyceride (B) and plasma urea (C) in the air breathing perch treated with varied concentrations of CHRE for five days. Each bar is mean ± SEM for eight fish. Statistical differences between fish groups were quoted after SNK test.
* P<0.05, **P<0.01

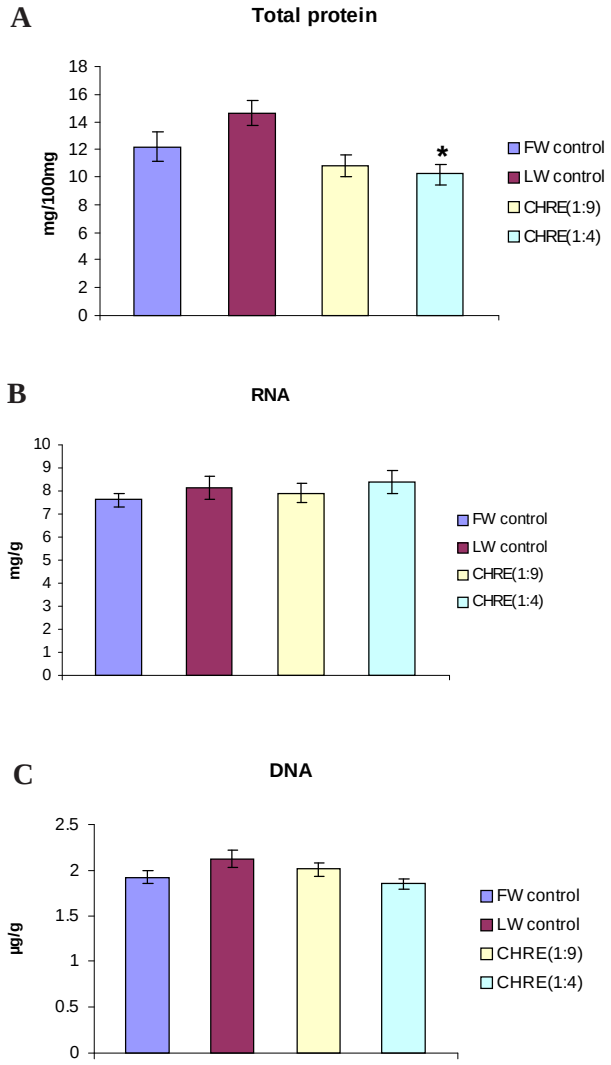

Fig. 2. Total protein (A), RNA (B) and DNA (C) in the air breathing perch treated with varied concentrations of CHRE for five days. Each bar is mean ± SEM for eight fish. Statistical differences between fish groups were quoted after SNK test.
* $P<0.05$

The increase in the plasma triglycerides in the perch treated with CHRE indicates an elevated energy demand during stress. It has been reported that lipogenesis in different tissues is sensitive to many chemical stressors (Gill et al., 1991, 1992; Khalaf-Allah, 1999). It is likely that increased mobilization of fuel substrates during CHRE intoxication forms a basis of metabolic adaptation during stress. It is also suggested that the increased cortisol availability may influence the lipid utilization in fish during CHRE-induced stress since there are reports on the decline in the total lipid in eel treated with lindane (Ferrando and Andreu-Moliner, 1991).

The urea turnover reflects the nitrogen balance. The increased urea content in the CHRE-exposed perch suggests an increased ureogenic potential. Teleosts excrete urea, and substantial production of urea requires amino acids as nitrogen donors (Walsh and Mommsen, 2001). Increased ureogenesis due to CHRE exposure suggests an overall increase in the protein catalytic function, which may be considered beneficial to the fish.

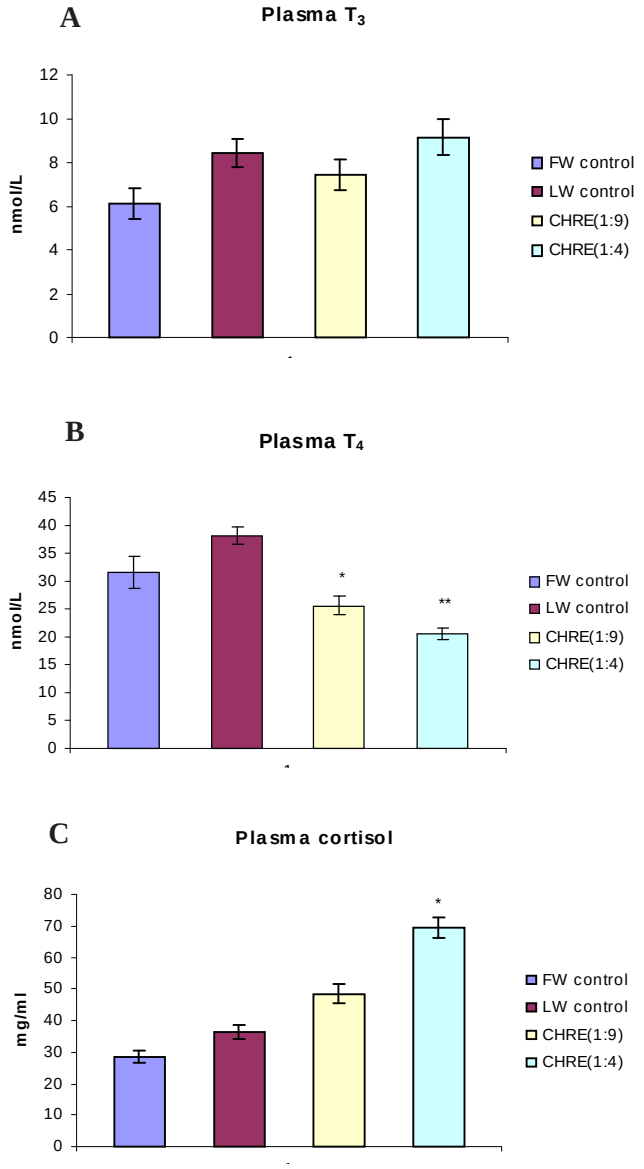

Fig. 3. Plasma T_3 (A), plasma T_4 (B) and plasma cortisol (C) in the air breathing perch treated with varied concentrations of CHRE for five days. Each bar is mean ± SEM for eight fish. Statistical differences between fish groups were quoted after SNK test.
* $P<0.05$, ** $P<0.01$

CHRE exposure has a proteolytic action as is evident in the reduction of liver total protein content. An elevated depletion of protein reserves in different tissues is an indication of stress since some preference of protein catabolism over anabolism may occur. Further, the protein metabolism in fish is highly vulnerable to the toxic action of toxicants including pesticides (Munshi et al., 1999; Khalaf-Allah, 1999). A decreased total serum protein has been reported in the freshwater teleost *Barbus conchonius* following endosulfan exposure (Gill et al., 1991). RNA and DNA content, important indicators of nucleic acid synthesis, did not change after CHRE exposure. The unaffected liver RNA and DNA content in the CHRE-exposed perch indicate a steady nucleic acid turnover in perch during CHRE-induced stress, although chemical stressors like kerosene had been shown to increase these nucleic acid contents (Peter et al., 2007).

Alkaline phosphatase is considered as the hallmark of lysosomal activity as it is engaged in the hydrolysis of protein. The increased AlP activity in the CHRE-exposed fish suggests a rapid mobilization of metabolites during stress. Similar results have been obtained in rosy barb exposed to pesticides and mercuric chloride, which increased the AlP activity in many tissues (Gill et al., 1990a, b). AST and ALT are transferases concerned with non-essential amino-acid metabolism and gluconeogenesis. The decrease in AST and increase in ALT activites in the CHRE-treated fish indicate elevated transamination process. This also suggests increased protein breakdown and gluconeogenesis in these fishes. The increased ALT activity also supports this view since increased transferase activity contributes to enhanced gluconeogenesis because amino acids can serve as substrates for gluconenogenesis (Walsh and Mommsen, 2001). A number of chemical stressors, including pesticides, have been shown to inhibit AST and stimulate ALT activities in liver and kidney tissues of rosy barb (Gill et al., 1990b) and the tilapia *Oreochromis niloticus* (Khalaf-Allah, 1999).

Overall, our study points to an altered metabolic regulation in the fish living in coconut husk retting ground. An up-regulated interrenal axis and a down-regulated thyroid axis support the hypothesis that these endocrines are involved in the process of stress tolerance in fish.

Acknowledgements

We thank University Grants Commission (UGC-MRP, F-30-205/2004, SR)) for financial support and UGC-SAP for support for infrastructural facility.

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